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Review

In the beginning: The establishment of the mammary lineage during embryogenesis

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ABSTRACT

The mammary primordium is comprised of an aggregate of immature, undifferentiated mammary epithelial cells and its associated mammary mesenchyme, a specialised tissue which harbours mammary-inductive capacity. The mammary primordium forms during embryogenesis as a result of inductive interactions between its two component tissues, the mammary mesenchyme and epithelium. These two tissues constitute a signalling centre that directs the formation of the mammary gland through a series of reciprocal mesenchymal–epithelial interactions. A rudimentary mammary ductal tree and stroma is formed prior to birth as a result of these interactions. The subsequent mammary outgrowths that arise upon hormonal stimulation during puberty originate from this rudimentary tissue. The initial appearance of the embryonic mammary primordium during embryogenesis represents the earliest morphological evidence of commitment to the mammary lineage. Classic tissue recombination studies of mouse mammary primordial cells have demonstrated that the epithelial cells are already functionally determined as mammary at the embryonic mammary bud stage. Recent studies have determined the molecular identity of the embryonic mammary cells by transcriptomic profiling and these have provided new insights into signalling components that mediate early embryonic mammary inductive signalling and lineage commitment. This review highlights what is currently known about the morphogenesis, function, and behaviour of embryonic mammary cells and examine current knowledge of the genetics underlying mammary cell fate and establishment of the mammary lineage during embryogenesis.

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1. Introduction

Mammary glands are one of the defining features of the Class Mammalia, meaning literally "of the breast". The term mammal was first used by Carl Linnaeus in his tenth edition of *Systema Naturae* who recognised the mammary gland as an important distinguishing

Abbreviations: EMT, epithelial–mesenchymal transition; ER, estrogen receptor; K14, Keratin 14; Krt, keratin; SMA, smooth muscle actin; TnC, Tenascin C.

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feature for the classification of animal types [1]. The fully differentiated mammary gland functions to produce and distribute milk and other substances to provide nutrition and immune support to neonates and young offspring. The functional potential of the mammary gland is achieved only after pregnancy and parturition but the initial formation of the gland occurs much earlier in the timescale of months in rodents and decades in humans. The mammary organ forms during embryogenesis and is first evident at E11.5 or Theiler stage 19 in the mouse [2]. In humans, by twelve weeks of gestation, the breast bud has formed [3]. A few notable studies using embryonic rabbit, rat, and human mammary tissues have been published and will be briefly discussed here, but the majority of developmental studies of the embryonic mammary gland have been performed in the mouse and thus form the basis of most data discussed in this review.

2. Overview of embryonic mouse mammary morphogenesis

2.1. Mammary line

Raised epidermal ridges known as "mammary lines" have been reported in rabbit, rat and human embryos and extend from the axilla to the groin along the lateral wall of the trunk [4]. The mammary line is obvious upon whole-mount analysis of the rabbit embryo and appears to fragment as the individual primordium form (Fig. 1) and [5]. The mammary lines span between and extend just beyond the limb buds and encompass the regions where the mammary primordium will subsequently form. Analysis of the rabbit mammary line using scanning electron micrography (SEM) detected cells with filopodia and lamellopodia, features characteristic of motile cells [5]. Analysis of mouse embryos with SEM failed to detect similar morphological structures in equivalent developmental stages [6]. The earliest stages of mouse mammary organ formation are characterised by the appearance of a pseudostratified epithelium which is detected upon histological sectioning or using confocal analysis (Fig. 2) of the presumptive mammary forming region between E10.5 and E11.0, stages at which no obvious localised thickenings on the ventral flank are detected [7,8]. Studies using reporter models and *in situ* hybridisation with probes, such as *Wnt10b*, have detected localised expression along an analogous region to the rabbit mammary line so that a molecular mammary line exists in the mouse [9]. The Wnt reporter mouse model, BAT-GAL which detects Wnt expression, and the Src homology 2 domain-containing inositol-5-phosphatase-reporter model, s-SHIP-GFP, which detects *s-SHIP* expression, both show expression along the mouse mammary line at sites where the mammary primordia will subsequently form from E10.5, suggesting that inductive events occur at a prior stage during mouse embryogenesis [10,11].

2.2. Mammary placode and bud stages

An elliptically shaped structure termed the mammary placode, comprised of an aggregate of epithelial cells, is the first morphological stage that is apparent upon light microscopy of unstained mouse embryos at a stage between E11.0 and E11.5 (Fig. 2B). This stage is followed by an early bud stage in which the primordium assumes a spherical shape (Fig. 2C). The mammary bud then increases in size until E14.5 (Fig. 2C–E). Results from calculating mitotic indices of the mammary-forming regions in both mouse and rabbit suggested that very little proliferation occurs at early bud stages of mammary formation and localised cell movements therefore are thought to largely contribute to the observed increase in cell numbers [8,12]. We have also observed a limited amount of cell proliferation within the embryonic mammary epithelium at E11.0–E14.5 stages when assessed using Ki67 staining (Kogata, Wansbury, and Howard, unpublished).

Male mouse mammary buds undergo an androgen-mediated destruction when the mammary mesenchyme condenses around the neck of the mammary bud, which occurs at E14.5 [13–17]. This event is a notable distinction between rodent and human mammary development where the mammary bud is retained and proceeds to form a small ductal outgrowth connected to the nipple. Some mouse strains and genetically modified mouse models retain the nipple in males, but how mammary bud destruction is avoided is not understood. Benign breast anomalies, such as Gynecomastia, an enlargement of the glandular component, may occur in males.

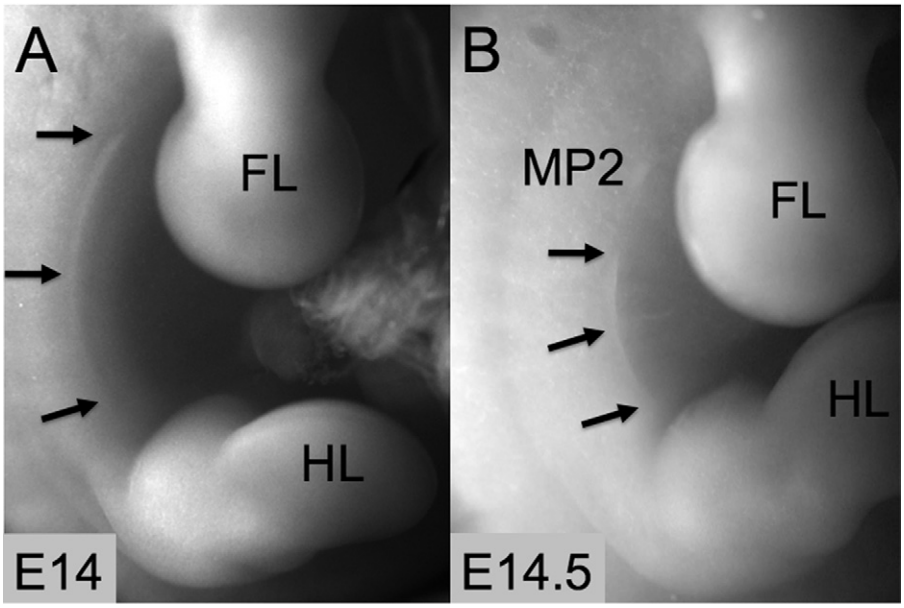

Fig. 1. Early stages of embryonic mammary development in rabbit. (A) The mammary line, a raised ridge of epidermal cells is visible between the fore and hind limbs spanning the regions where the five pairs of mammary primordia will subsequently form in a rabbit stage E14.0 embryo. (B) The mammary line appears to fragment so that individual mammary primordium form at sites along the former line in a rabbit stage E14.5 embryo. FL, forelimb; HL, hindlimb, MP2, mammary primordium 2.